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The increased activity of plasma manganese superoxide dismutase in tardive dyskinesia is unrelated to the Ala-9Val polymorphism

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Abstract

That tardive dyskinesia (TD) may have its origins in free-radical toxicity has stimulated investigations into one enzyme important in the control of oxidative free radicals: superoxide dismutase (SOD). The manganese-containing form of this enzyme (MnSOD) is the major superoxide scavenger in mitochondria; a weak association between a functional genetic polymorphism (Ala-9Val) in the mitochondrial targeting sequence (MTS) of this enzyme and TD has been reported in a Japanese population. We have undertaken to determine both the plasma activity of MnSOD and the association of the Ala-9Val polymorphism in a well-matched series of male Chinese schizophrenic patients with ($n=42$) and without ($n=59$) TD, and normal male controls ($n=50$). MnSOD activity was elevated in the TD subjects over those without TD ($P<0.05$) and normal controls ($P<0.05$), an effect that was independent of age, age at first antipsychotic treatment, drug dosage and duration of illness. A significant positive correlation between total AIMS score and MnSOD activity was also observed ($P<0.0001$). No significant reduction in the frequency of the Ala allele was observed in the TD group (0.14) below non-TD (0.18) or control subjects (0.17); nor was there any relationship between MnSOD activity and the polymorphism. There was no difference between the mean AIMS scores for the two genotypes (V/V and A/V) in the TD group. We conclude that while we have further evidence of a disturbance in the mechanisms regulating oxidative free radicals in TD, this effect is not under the control of the genetic polymorphism investigated here. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Manganese superoxide dismutase (MnSOD); Polymorphism; Tardive dyskinesia; Schizophrenia; Pharmacogenetics; Association study

1. Introduction

Tardive dyskinesia (TD) is a serious adverse drug effect, characterised by the delayed appearance of involuntary movements, affecting roughly 25% of schizophrenic patients chronically treated with neuroleptic drugs (Najib, 1999; Glazer, 2000). Although the mechanism of TD is not fully understood, several lines of evidence suggest that increased oxidative stress, an imbalance between free radical metabolism and the antioxidant defence system, plays an important role in the development of TD (Cadet and Kahler, 1994; Elkashef and Wyatt, 1999).

Previous studies have reported elevated levels of measures of lipid peroxidation in the cerebrospinal fluid (CSF) in patients with TD as compared to those without (Lohr et al., 1990), and increased lipid peroxidation both in CSF and plasma was significantly correlated with the severity of TD (Lohr et al., 1990; Peet et al., 1993; Brown et al., 1998). Furthermore, the efficacy of treatment of TD with vitamin E and other antioxidants further indicated the involvement of oxidative free radicals in the pathogenesis of this side effect (reviewed by Elkashef and Wyatt, 1999).

The first line antioxidant defence enzymes, the superoxide dismutases (SODs), play a critical role in protecting cells against damage from free radicals. SODs consist of three isoforms, including intracellular SOD (Cu/ZnSOD), extracellular SOD (FeSOD) and intramitochondrial SOD (MnSOD). There are reports of both increased and decreased SOD activity in first episode or chronic patients with schizophrenia (Abdalla et al., 1986; Reddy et al., 1991; Mukherjee et al., 1996; Khan and Das, 1997; Yao et al., 1998), which have been

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interpreted as indicating increased oxidative stress in the pathogenesis of schizophrenia. Yamada et al. (1997) further reported decreased erythrocytic SOD activity in chronic neuroleptic-treated schizophrenics with TD as compared to patients without TD or with questionable TD, and the severity of TD was negatively correlated with SOD activity either in erythrocytes (Yamada et al., 1997) or in CSF (Tsai et al., 1998). In addition, Loven et al. (1996) found significantly increased MnSOD activity in the frontal and temporal lobes of chronic neuroleptic-treated psychotic patients as compared with controls, but no change in Cu/ZnSOD, interpreted as a response to increased superoxide radical production due to a neuroleptic-induced increase in CNS dopamine turnover.

MnSOD is an intramitochondrial enzyme that scavenges superoxide anions generated in mitochondrial energy metabolism. The human MnSOD gene is located in the long arm of chromosome 6 (6q25), which has also been implicated as a candidate region for schizophrenia (Moldin and Gottesman, 1997). Up to now, four functional polymorphism sites on the MnSOD gene have been reported. Most studied is the Ala-9Val (GCT/GTT) polymorphism in exon 2 of the mitochondrial targeting sequence (MTS), which is probably the first known human polymorphism of the MTS of proteins. The Ala-9Val substitution may lead to misdirected intracellular trafficking and affect the mitochondrial targeting rate of MnSOD, resulting in mitochondrial damage (Shimoda-Matsubayashi et al., 1996; 1997; Rosenblum et al., 1996). In addition, Borgstahl et al. (1996) reported an amino acid substitution Ile58Thr that affects the stability of the protein; however, the 58Thr variant (at nucleotide position 5777 of exon 3) was not found in a study of 63 German Parkinson's disease patients (Grasbon-Frodl et al., 1999).

Previous studies have reported that the variant-9Ala allele increases risk of breast cancer (Ambrosone et al., 1999; Mitrunen et al., 2001), and associates with sporadic motor neuron disease (Van Landeghem et al., 1999a), exudative age-related macular degeneration (Kimura et al., 2000) and Parkinson's disease (Shimoda-Matsubayashi et al., 1996), which are all related to free radical pathogenesis. Most recently, Hori et al. (2000) have found a small but significant increase in the wild-type Val allele in Japanese schizophrenic patients with TD as compared to patients without TD, suggesting the-9Ala allele may act protectively against free radical damage in TD.

However, the relative allelic frequencies of this polymorphism varies between different ethnic groups (Van Landeghem et al., 1999b). Thus, the Ala frequency was significantly lower in an Asiatic population (Chinese Singapore: 30%) than in most European populations (41–62%). Furthermore, there are studies indicating a wide variation of 2–50% in the prevalence of TD depending on ethnicity (Swartz et al., 1997), with about 9.3% in a schizophrenic Chinese population (Chiu et al., 1992), which may be attributed to the difference in genetic vulnerability to the development of TD in different ethnic populations. These observations, an association of the Ala-9Val polymorphism with TD and ethnic variability of both this allele and prevalance of TD, prompted us to investigate whether the Ala-9Val genotypes of the MnSOD gene may play a role in the development of TD in Chinese Han schizophrenic patients. Moreover, we aimed to determine if and how the functional polymorphism site may influence MnSOD activity in plasma, and whether variations in this activity may relate to the presence of TD.

2. Subjects and methods

2.1. Clinical subjects and assessment

The male patients with schizophrenia were all chronic and long-term inpatients referred to the Department of Psychiatry, YangZhouWuTaiShan Hospital (JiangSu Army Psychiatric Hospital), JiangSu Province, P.R. China. They had been diagnosed with schizophrenia and acutely treated in a local army psychiatric hospital and subsequently transferred for further treatment. All patients have complete medical records from first admission. Clinical and demographic information was obtained from medical records and interview with patients. A total of 326 patients had been treated with antipsychotic agents for longer than 8 years (30.45± 8.46 years; range 9–52 years). All patients met criteria for a diagnosis of schizophrenia as re-determined by senior psychiatrists (ZJZ, XBZ, WWS) according to DSM IV (American Psychiatric Association, 1994) based on clinical examination and medical records.

All patients were assessed for tardive dyskinesia (TD) by the Abnormal Involuntary Movement Scale (AIMS) employing a standard evaluation method (Guy, 1976) during November, 2000. In the AIMS, dyskinesia was observed in different regions of the body including the facial and oral regions, the extremities, and the trunk. Severity ratings were made along a 5-point ordinal scale from 0 to 4 (none, minimal, mild, moderate and severe), and diagnosed presence or absence of TD according to the research diagnostic criteria for TD (Schooler and Kane, 1982) if there were at least moderate or severe abnormal involuntary movements in one or more of the seven body areas examined, or at least mild movements in two or more areas. Initially, the patients were diagnosed as having TD ($n=48$) or being without TD ($n=278$), respectively. Sixty-five out of 278 patients without TD have been further matched by age, age of onset, age at first antipsychotic treatment, duration of illness, time of antipsychotic treatment, total anti-

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psychotic exposure and current daily dose (chlorpromazine equivalent dose) to the TD group. The patients with TD and the well-matched group without TD were further evaluated for TD by AIMS twice more during January, 2001 and March, 2001. Eventually, a total of 42 patients with persistent TD defined the TD group, and 59/65 patients consistently without TD as the no TD group. All of the patients were also rated using the Brief Psychiatric Rating Scale (BPRS) for evaluating psychiatric symptoms one day before collection of samples.

All patients had received stable doses of antipsychotic drugs during the previous 3 months. Antipsychotics prescribed were currently chlorpromazine (40.6%), clozapine (36.6%), combination of chlorpromazine and clozapine (9.9%), fluphenazine (6.3%), sulpiride (5.6%) and haloperidol (1%), and a total 66 out of 101 patients had received multiple antipsychotic drugs in their lifetime. In addition, 19 patients were receiving adjunctive anti-cholinergic treatment.

No participant patients had a history of substance abuse or alcoholism, and all were without additional neurological disease and without other physical diseases such as diabetes. Most patients had no access to cigarettes since admission; five patients had one-three cigarette/per day, but had not smoked for at least 3 days prior to collection of whole blood. Other clinical characteristics of the patients are shown in Table 1. The controls were all healthy male volunteers ($n=50$, mean age$=56.4\pm7.9$ years, range 36–75) with no individual and family history of mental illness who were unrelated to the patients but shared the same demographic and social characteristics and occupation as the patients. The project was carried out with the approval of the local ethical committee and was in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants. All subjects were of Chinese Han ethnic origin and originated from JiangSu province.

2.2. Experimental work

2.2.1. Measurement of MnSOD activity in plasma

Whole venous blood samples were taken into tubes containing EDTA at approximately 6:30 am following overnight fasting after final clinical assessment. 5ml whole blood has been kept at −80 °C for extraction of genomic DNA (see Section 2.2.2.). After centrifugation at 2500 *g* at 4 °C for 5 min, plasma supernatants were collected and immediately stored at −80 °C prior to assay. The samples were processed randomly and blind to diagnosis.

MnSOD activity was measured in plasma (Chiou and Hu, 1999) using a standard assay involving spectrophotometric determination of the inhibition of superoxide-induced formation of nitrite from hydroxylamine, as described by Oyanagui (1984). Xanthine-xanthine oxidase provided the superoxide source and KCN was used to inhibit CuZnSOD. In a typical assay, an aliquot of 30 µl plasma was used to measure SOD activity. One unit of SOD activity was defined as that amount that would inhibit nitrite reduction by 50% (NU/ml plasma) under the assay conditions. The inter- and intra-assay coefficients of variation for SOD activity are 3.7% ($n=4$) and 2.6% ($n=4$).

2.2.2. Genetic analysis

Genomic DNA was extracted from 5-ml aliquots venous blood using Nucleon BACC Kits (Nucleon-Biosciences, Scotland, UK) according to the manufacturer's recommendations. The genotyping was performed strictly blind to the clinical status of patients. A genomic DNA fragment corresponding to the MTS in the human MnSOD sequence (Genebank accession number D83493) was amplified by the polymerase chain reaction (PCR) method, using the following specific primers: forward 5′-AGC CCA GCC TGC GTA GAC 3′, reverse 5′-TAC TTC TCC TCG GTG ACG-3′ as previously described by Grasbon-Frodl et al. (1999) in a total volume of 25 µl, containing about 50 ng of genomic DNA, 10 pmol/µl of each primer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1X PCR buffer containing 5 mM Tris–HCl (pH8.0 at 25 °C), 10 mM NaCl, 0.01 mM EDTA, 0.1 mM DTT, 5% glycerol, 0.1% Triton© X-100, and 1.5 units of Taq DNA polymerase (Promega, UK). The PCR reaction conditions involved an initial denaturation at 94 °C for 6 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 2 min, followed by extension at 72 °C for 10 min. The resulting 246-bp PCR product was digested with the restriction endonuclease BsaW I for 3 h at 60 °C according to the manufacturer's recommendations (New England Biolab, UK), and digestion products were analysed following electrophoresis in 12% polyacrylamide gel stained with silver nitrate. Restriction enzyme digestion results in a 246 bp product (allele 1 Ala-9) or 164 bp and 82 bp products (allele 2 Val-9).

2.3. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 10.0. for Windows. Testing for normality was accomplished by normality plots and K-S (Lilliefors) tests. All MnSOD activity data showed normal distributions. The χ^2-goodness-of-fit test was used to test the distribution of genotypes and allele frequencies for deviations from Hardy–Weinberg equilibrium. Chi-square (or Fisher's exact test) was used to compare allele and genotype frequencies between patient groups and normal controls. The *t*-test for independent samples and analysis of variance (ANOVA) were used to compare age, age of onset, duration of illness, age of first antipsychotic

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Table 1
Clinical demographic features of schizophrenic patients with TD and without TD

Subject groups	TD ($n=42$) mean±S.D.	No TD ($n=59$) mean±S.D.
Age (years)	56.1±10.4	54.8±6.9
Age of onset of schizophrenia (years)	24.1±5.4	24.2±4.3
Duration of illness (years)	32.2±9.7	30.8±6.2
Age at first neuroleptic treatment (years)	25.1±7.2	24.5±4.4
Duration of untreated psychosis (years)	0.98±2.45	0.53±1.51
Length of antipsychotic treatment (years)	30.6±10.9	30.4±6.7
Current antipsychotic day dose (mg chlorpromazine equivalents)	324.3±141.1	356.6 ±120.3
Lifetime antipsychotic doses(g chlorpromazine equivalents)	3344±1872	3993±1915
Family history of mental disorders	Y/N: 11/31	Y/N: 13/46
Rating scale: BPRS total	51.2±7.8	49.6±7.3

treatment, period of antipsychotic treatment, lifetime antipsychotic dose, current antipsychotic dose, and the Mann–Whitney test for difference in total AIMS scores and total BPRS scores. Analysis of covariance (ANCOVA) was performed to compare plasma MnSOD activity between the subject groups, as well as between genotype groups, using age, duration of illness and lifetime antipsychotic dose as covariates. Values are give as mean±standard deviation (SD). Significance levels are for two-tailed tests; P values <0.05 were regarded as significant. Correlation between MnSOD activity and clinical parameters were examined by bivariate correlation (Pearson or Spearman correlation coefficients). Relationship between total AIMS scores and genotypes and MnSOD activity in the patients with TD group were examined either by multivariate regression by stepwise multiple regression or by ANCOVA using prominent confounding clinical features (e.g. age, age of first antipsychotic treatment, duration of illness and lifetime neuroleptic dose) as covariates.

3. Results

3.1. Clinical demographic characteristics

Clinical demographic characteristics of schizophrenic patients are shown in Table 1. All clinical features between the TD and without TD groups were well-matched. There were no significant differences in terms of age, age of onset, age of first antipsychotic treatment, duration of illness, period of antipsychotic treatment, lifetime antipsychotic dose, current antipsychotic dose in chlorpromazine equivalents, degree of severity of psychotic symptoms (total BPRS scores) and family history between the two groups.

3.2. Allele and genotype frequencies

Allele and genotype frequencies of the subjects for the Ala-9Val polymorphism in the MnSOD gene are showed in Table 2. The genotypic distributions in patients as a whole group ($\chi^2=3.83$, d.f.$=2$, $P=0.15$), the patients with TD ($\chi^2=1.15$, d.f.$=2$, $P=0.56$) and the patients without TD ($\chi^2=2.73$, d.f.$=2$, $P=0.26$) as separate groups, and normal controls ($\chi^2=2.05$, d.f.$=2$, $P=0.36$) were consistent with the Hardy–Weinberg equilibrium. In the present investigations, we found no Ala/Ala genotype in a total of 151 subjects. There were no significant differences in the distribution of the allelic frequencies and genotypes between schizophrenic patients and healthy controls ($\chi^2=0.21$, d.f.$=1$, $P=0.88$; $\chi^2=0.03$, d.f.$=1$, $P=0.87$, respectively), and between the patients with TD and the patients without TD and normal controls ($\chi^2=0.14$, d.f.$=2$, $P=0.93$; $\chi^2=0.57$, d.f.$=2$, $P=0.78$, respectively), although slightly higher frequencies of the Val/Val genotype and the Val allele were seen in the patients with TD as compared to the patients without TD. There were no significant differences in any clinical demographic characteristics and scores of clinical assessment between the two genotypic groups in the patients or within the TD group (data not shown).

3.3. The activity of MnSOD in plasma between the subject groups

Table 3 shows the comparison of plasma MnSOD activity between patient groups and normal controls as well as between genotypic groups in schizophrenic patients and in the TD group. There was a significant difference in MnSOD activity in plasma between the three subject groups (ANCOVA with age as covariate, $F=10.9$, d.f.$=3$, $P=0.0001$); post hoc testing showed a significantly higher level of MnSOD activity in the patients with TD as compared to the patients without TD ($P<0.05$) and to normal controls ($P<0.05$). No significant difference in the activity was found between two genotypic groups within all patients (ANCOVA with age as covariate, $F=1.7$, d.f.$=2$, $P=0.1$) as well as within the patients with TD group (ANCOVA with age as covariate, $F=1.5$, d.f.$=2$, $P=0.2$), although the mean MnSOD activity was higher in the Val/Val genotype group as compared to the Ala/Val genotype group

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Table 2
Genotype and allele frequencies of the Ala-9Val polymorphism in schizophrenic patients and normal controls

	Allele frequency		Genotype distribution					
	−9Ala	−9Val	Ala/Ala		Ala/Val		Val/Val	
Subject groups (n)	Frequency	Frequency	N	Frequency	N	Frequency	N	Frequency
Patients (101)	0.16	0.83[a]	0	0 (0.03)	33	0.33 (0.27)	68	0.67 (0.70)[b]
Controls (50)	0.17	0.83[a,c]	0	0 (0.03)	17	0.34 (0.28)	33	0.66 (0.69)[b,d]
With TD patients (42)	0.14	0.86[c]	0	0 (0.02)	12	0.29 (0.25)	30	0.71 (0.74)[d]
No TD patients (59)	0.18	0.82[c]	0	0 (0.03)	21	0.36 (0.29)	38	0.64 (0.68)[d]

The expected genotype distribution according to a Hardy–Weinberg equilibrium is shown in parentheses.
[a] Schizophrenic patients versus normal controls ($\chi^2=0.21$, d.f. = 1, $P=0.88$).
[b] Schizophrenic patients versus normal controls ($\chi^2=0.03$, d.f. = 1, $P=0.87$).
[c] TD versus no TD and controls ($\chi^2=0.14$, d.f. = 2, $P=0.93$).
[d] TD versus no TD and controls ($\chi^2=0.57$, d.f. = 2, $P=0.78$).

Table 3
Comparison of MnSOD activity in plasma between the subject groups

	MnSOD activity (NU/ml plasma) (mean±S.D.)		
[a]TD vs. no TD vs. controls	102.3±11.3	50.6±7.2	57.8±5.4
[b]Val/Val vs. Ala/Val in all patients	77.5±8.8	60.8±9.9	
[c]Val/Val vs. Ala/Val in TD patients	111.8±13.8	78.5±18.4	

[a] ANCOVA $F=10.9$, d.f. = 3, $P=0.0001$; post hoc testing: TD vs. no TD $P<0.05$; TD vs. controls $P<0.05$.
[b] ANCOVA $F=1.7$, d.f. = 2, $P=0.1$; schizophrenics with Val/Val genotype vs. those with Ala/Val genotype.
[c] ANCOVA $F=1.5$, d.f. = 2, $P=0.2$; schizophrenics/TD with Val/Val genotype vs. those with Ala/Val genotype.

in the patients with TD. Furthermore, MnSOD activity correlated positively with total AIMS scores in patients with TD (Spearman: $r=0.71$, $P=0.0001$), but not with other clinical features, consistent with the previously mentioned results.

3.4. Relationship between MnSOD genotypes, MnSOD activity and total AIMS scores

In order to analyse the possible contribution of the MnSOD genotype and clinical features and MnSOD activity to the severity of TD, we performed stepwise multiple regressions. When the MnSOD genotype, MnSOD activity, age, age of first antipsychotic treatment, lifetime antipsychotic dose, duration of illness were entered into regression models as independent variables, the total AIMS score was significantly associated with plasma MnSOD activity (beta = 0.66, $t=5.57$, $P=0.0001$), but not with MnSOD genotype and other clinical features. ANCOVA also indicated no significant difference in mean total AIMS scores between genotype groups in the patients with TD.

4. Discussion

In the present study, we found no significant differences either in allele or in genotype in the Ala-9Val polymorphism between schizophrenics and control subjects or between patients groups with or without TD. Moreover, genotype did not significantly affect the activity of MnSOD in plasma in the patient groups. In addition, genotype did not relate to total AIMS scores in the patients with TD. However, there was a significant increase in the activity of MnSOD in plasma in the patients with TD as compared with the patients without TD and normal controls. The increased activity was positively correlated with AIMS scores within the TD group.

In the present study, the chronic male patients with schizophrenia were recruited from the long-term wards of an army psychiatric hospital. They were all of JiangSu origin located in Southeast China, which limited the influence of heterogeneity of population structure in producing a false positive result in such a case-control association study. Moreover, all patients have complete medical records from first admission, providing an ideal population of chronic medicated patients with schizophrenia, permitting both well-matched patient groups, and the opportunity to analyse potential confounding effects on the severity of TD such as lifetime neuroleptic dose and age of first antipsychotic treatment. Importantly, the occurrence and diagnosis of TD was assessed by AIMS score and confirmed at follow-up assessment over five months. Thus, only patients with persistent TD were included in this study as the TD group. Furthermore, the group without TD was well-matched for all clinical characteristics to those with TD.

In the present study, 42 patients with persistent TD out of 326 chronic schizophrenic patients in the hospital

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indicated a 12.9% prevalence of TD in a Chinese Han population, which is consistent with the reports of Chiu et al. (1992) and others (Pandurangi et al., 1995). The Ala-9Val polymorphism of MnSOD varies in different ethnic populations, with lower (11–30%) Ala allelic frequencies reported in Japanese (Matsumine et al., 1997; Hori et al., 2000) and Chinese populations than the 41–62% in Europeans (Van Landeghem et al., 1999b). The present study is consistent with this, finding a 17% frequency in Chinese Han male controls.

There are some inconsistencies in the interpretation of the role of the Ala allele in disease processes. Studies in parkinsonism (Matsumine et al., 1997; Shimoda-Matsubayashi et al., 1996), motor neuron disease (Van Landeghem et al., 1999a), macular degeneration (Kimura et al., 2000) and breast cancer (Mitrunen et al., 2001) all associate an increase in the Ala variant with vulnerability to toxic processes, while the increased Val/Val genotype reported in TD (Hori et al., 2000) is interpreted as indicating a protective effect of the Ala allele. The polymorphism of the MTS of MnSOD is believed to affect the targeting of the protein to the mitochondria. The above studies suggested that the processing efficiency is influenced by the allele in such a manner that MnSOD with the Val polymorphism is less efficiently transported into mitochondria. It is possible that a more efficient MnSOD protein produces excess hydrogen peroxide that in turn may react with ferrous iron to form more cytotoxic hydroxyl radicals, resulting in greater free radical damage.

Although Hori et al. (2000), in the first report of the Ala-9Val polymorphism in a schizophrenic population, found a slight but significant increase in the Val/Val genotype in patients with TD, they did not find an association between genotype and the severity of TD or relate genotype with MnSOD enzyme activity. In the present study, we found no significant difference in allele and genotype frequencies between schizophrenic patients with and without TD, although there was a slight increase in the Val/Val genotype in the TD group (0.71 vs. 0.64). Again, no significant association was found between AIMS score and genotype in the patients with TD. Thus we have been unable to confirm the findings of Hori et al. (2000) in a Chinese Han population.

However, the present work only tested this polymorphism in a male population. Female sex has previously been considered a risk factor for TD, although this has been disputed (reviewed by Owens, 1999). It is notable that Hori et al. (2000) did not observe any influence of sex on their results. The persistent nature of the disorder in our TD group and the careful matching of a comparison group of patients without dyskinesias would be expected to provide a clearer distinction of any genetic risk factor associated with TD. However it is conceivable that other genetic factors associated with TD, such as those relating to polymorphisms of the dopamine D3 and the 5-HT2C receptors (e.g. Steen et al., 1997; Segman et al., 2000; Zhang et al., 2002), may have introduced variables that mask any effect of this MnSOD polymorphism. There is, of course, a inherent difficulty in determining the genetic influences on a disorder that is clearly induced by an environmental factor, in this case antipsychotic drug treatment, exposure to which varies substantially between patients. One further confounding factor is that this treatment can itself be protective; certainly there is evidence that clozapine can diminish the symptoms of TD (reviewed by Sachdev, 2000). Although several patients in the present study were receiving clozapine, the prescribing of this drug between the TD and non-TD groups was not significantly different, and no indication of any genetic association emerged when these patients were removed from the analysis (data not shown).

However, MnSOD remains a potential contributor to the pathogenesis of TD. MnSOD knockout mice exhibit pathologic phenotypes including degeneration of neurones in the basal ganglia and brainstem and progressive motor disturbances, as well as extensive mitochondrial injury within degenerating neurones (Lebovitz et al., 1996; Murakami et al., 1998; Williams et al., 1998; Melov et al., 1998). These observations consistently indicate that mutation of the MnSOD gene causes increased susceptibility to oxidative mitochondrial injury in the CNS resulting in motor dysfunction. Interestingly, Przedborski et al. (1998) found that MnSOD removal may have a more direct and pronounced physiological effect than knocking out the Cu/ZnSOD gene (Bruijn et al., 1998). Other functional polymorphism sites of the MnSOD gene may contribute to the susceptibility to TD and deserve further investigation. In addition to the very rare Ile58Thr polymorphism, Matsumine et al. (1997) reported both a neutral polymorphism (GGA/GGG) in codon 157Gly in exon 5 and Ser3Arg (AGC/AGA) in exon 2, although the functional effects of this polymorphism have not been fully investigated.

It is notable that we have found increased MnSOD activity in the schizophrenic patients with TD as compared to those without TD; enzyme activity is further positively correlated with the severity of TD determined by total AIMS scores. Again, groups were matched and controlled for clinical characteristics and other potentially confounding effects on enzyme activity such as smoking and diet. Nor was there any effect on activity from age, disease duration and antipsychotic drug dosages. However, previous studies indicated clozapine and chlorpromazine can act as powerful antioxidants (Jeding et al., 1995; Dalla-Libera et al., 1999) although results are conflicting. By contrast, haloperidol is a powerful pro-oxidant, generating reactive oxygen species and inducing oxidative damage in vitro and in vivo

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(Fang et al., 1996; Jeding et al., 1995). However, only one patient was receiving haloperidol. No difference was apparent between the patient groups in the patterns of antipsychotic drugs in either lifetime treatment or current therapy, nor could we find any effect of current drug treatment on SOD activity. Thus we were unable to ascribe the differences in SOD activity between patient groups or individual patients to any specific factors other than the severity of TD.

Increased MnSOD activity may play an essential role in protection against the development of TD induced by free radicals. However, when SOD activity increases without compensatory increases in glutathione peroxidase and/or catalase, excess hydrogen peroxide converts into the highly reactive hydroxyl radical and eventually results in free radical chain reactions. On the other hand, inadequate SOD activity can also lead to increased oxidative stress by from excess superoxide (Halliwell and Gutteridge, 1989). Although our measure of plasma MnSOD cannot be directly extrapolated to that in the CNS, there is evidence that peripheral indicators of free radical activity are correlated with changes in the central nervous system (Lohr, 1991; Jenner, 1994; Reddy and Yao, 1996; Mahadik and Mukherjee, 1996).

In conclusion, the increase in MnSOD activity that we identify in plasma in patients with TD is certainly indicative of a dysfunction of free radical mechanisms, although it cannot be distinguished whether this increase is a potentially protective compensatory effect or a pathogenic factor. Despite the earlier report of an association of TD with the Ala-9Val MnSOD polymorphism, we have been unable to confirm this finding nor relate it to the abnormal activity of this enzyme in TD. However, since changes in enzyme activity may relate to effects on transcriptional processes, it is possible that polymorphisms in e.g. the promotor region of the MnSOD gene may contribute to genetic susceptibility to TD, as is found with the 5-HT2C receptor gene (Zhang et al., 2002).

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